incorporated by reference in the present specification at page 12, lines 7-9. Accordingly, it does not constitute the addition of new matter.

Objection under 35 U.S.C. § 132

The Examiner objected to the amendment filed January 2, 2002, on the basis that it allegedly introduces new matter into the disclosure with the amendment of the paragraph at page 16, lines 27-34. The amendment concerned switching the assignment of the marks for HRP activity of intact cells and cytosol in Figure 4. It would be clear to one of ordinary skill in the art that the amendment represents correction of a typographical error. Example 3, which describes the experiments and results presented in Figure 4, states in the title that PCI (photochemical internalization) induces the release of a large fraction of the endocytosed molecule. It is also indicated at line 13 on page 22 that PCI induces the release of a large fraction of endocytosed HRP into the cytosol and refers to this being more than 60%. With the amendment, the cytosol graph of Fig. 4 is shown with open circles. This correlates to increasing levels of HRP in the cytosol with light exposure, peaking at more than 60% at 60 seconds. Thus, it is clear from the text as filed that the open circles are the data points for the cytosol. The closed circles, in contrast, show HRP levels staying constant and decreasing with light exposure. If the closed circles represented cytosol, this would be inconsistent with the description that photochemical internalization (caused by light exposure) induces the release of a large fraction of endocytosed HRP into the cytosol. Accordingly, it would be clear to one of skill in the art that the amendment of the paragraph at page 16, lines 27-34 is correction of a typographical error.

The 35 U.S.C. §112, Paragraph 1, Rejections of the Claims

In the Office Action mailed April 24, 2001, the Examiner rejected claims 1-11 under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. Insofar as this rejection may be reinstated in response to the present amendments of claims 1 and 2, it is respectfully traversed.

The Examiner stated that absent any definition for "part thereof" of an antigenic molecule, a "part thereof" could encompass even single amino acids. Claims 1-11 recite a method of expressing an antigenic molecule on the surface of a cell involving introducing a molecule into the cell cytosol by photochemical internalization, wherein said molecule or a part thereof of sufficient size to generate an immune response is subsequently presented on the surface of said cell. The specification discloses at page 7, lines 30-32, as is well known in the art, that to generate an immune response, a peptide must, for instance, be greater than 5 or greater than 10 or 20 amino acids. Thus, the claims do not encompass a process involving presenting single amino acids on the cell surface. Accordingly, the claims comply with the written description requirement of 35 U.S.C. § 112, paragraph 1.

Claims 1-11 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. This rejection is respectfully traversed.

To support the enablement rejection, first the Examiner asserts that MART-1 cannot be considered a well-known vaccine antigen. In response, Applicant points out first that the claims are not directed to a vaccine or to a method using MART-1 in particular. The claims are directed to a method of expressing an antigenic molecule on the surface of a cell. Regardless of whether MART-1 is a well known vaccine, the references cited at pages 5-6 of the response to the previous Office Action show, as argued previously, that MART-1 is an antigen: it elicits an immune response after in vivo or in vitro presentation. However, the Examiner states that the instant rejection is not related to the antigenicity of any particular antigen (page 3, first paragraph of present Office Action). Applicant agrees, and contends that provided stimulation of an immune response can be achieved with MART-1 or any other antigenic molecule or part thereof, the present claims are enabled. Whether MART-1 or any other specific molecule stimulates a large immune response or a small immune response is irrelevant to the patentability of the present claims.

The Examiner next asserts in support of the enablement rejection that neither cell surface expression nor antigenic presentation has been shown and that the stimulation of an immune

response has not been shown (Office Action page 3, first full paragraph). The Examiner disputes Applicant's contention that Example 2 discloses cell surface expression of MART-1 (Office Action, page 4, first full paragraph). The Examiner asserts that Example 2 does not disclose all the necessary controls.

In response, Applicant encloses a Declaration under Rule 132 of one of the inventors, Anders Hogset. In the Declaration, Dr. Hogset discloses the results of experiments performed as described in Example 2 except that the experiments were performed with or without the MART-1 peptide (paragraph 4 and Figure 1 of the Declaration). Cells were treated with a photosensitizing agent, then exposed to MART-1 (+ MART-1) or not exposed to MART-1 (- MART-1), and then exposed to light. 18 hours after light exposure, the cells were exposed to cytotoxic T-lymphocytes (CTL) and lysis was assayed. The experiment showed that light-dependent cytotoxicity occurred only with cells exposed to MART-1 (Figure 1 of the Declaration).

Thus, with Example 2 and the Declaration, there are four components in the assay that are relevant, and the following permutations of these components have been tested.

	Photosensitizer	MART-1	Light	CTL
1	+	+	+	+
2	+	+	-	+
3	+	+	+	-
4	+	-	+	+
5	+	-	-	+

The first three rows are derived from the experiments of Example 2. The fourth and fifth rows are derived from the experiment detailed in the declaration.

The first row experiment showed high Cr release (high cell killing), with the second row showing reduced Cr release, and the third row illustrating the background or zero value. The fourth and fifth rows show very little Cr release. This allows the following conclusions to be drawn: (i) CTL must be involved in cell destruction (rows 1 and 3), (ii) light is necessary (rows 1 and 2), and (iii) MART-1 is required for CTL-mediated killing (rows 1 and 4). From these

conclusions, it can be established, as Dr. Hogset declares, that photochemical treatment results in MART-1 internalization, processing and presentation on the surface of the cells in a form such that immune effector T cells are able to recognize and eliminate those cells (paragraph 4 of the Declaration).

This cell killing is dependent on the presence of the MART-1 peptide. There is virtually no CTL-dependent cell killing without the MART-1 peptide regardless of whether the cells are illuminated or not. Addition of the MART-1 peptide induces a modest level of cell killing without illumination, but illumination increases the cell killing substantially (see the Declaration).

In view of what is known about this process, this dependence on the MART-1 peptide indicates internalization, processing and presentation on the surface of the cell. The CTL cell clone used (Kirkin et al., 1999, Cancer Immunol. Immunother. 48:239-246 (enclosed herewith)) has been shown to be specific for the MART-1/HLA-A2 combination and would therefore only kill cells containing a MART-1 peptide presented on the cell surface and binding correctly in the peptide binding groove in HLA-A2. MART-1 peptide presented in another context would not be recognized by this CTL clone and therefore the cells which are killed must contain the MART-1 peptide appropriately bound to HLA-A2 and presented at the surface. This correct association can only occur via the normal processing pathway after internalization of the MART-1 peptide. It is known that class 1 MHC molecules are not transported to the cell surface unless they are "loaded" with peptide. Furthermore, correct presentation of the peptide requires the formation of an 8-9 amino acid fragment. The MART-1 peptide used in the experiment is 16 amino acids in length and thus for presentation to occur must have been proteolytically processed inside the cell.

Finally, the types of cells used in the experiment lack mechanisms which allow endocytosed molecules (e.g. MART-1) to be transferred to the cytosol and ultimately expressed on the surface, unlike dedicated antigen presenting cells. This is clear from the experiments in which MART-1 when added to the cells failed to induce cell killing unless coupled with photochemical internalization (PCI) (row 2 experiment). PCI however provides a means for cytosolic transfer in such cells that are normally not able to pass endocytosed antigens on to the

cytosol and therefore are normally not able to present antigens on MHC Class I. As soon as the antigen is in the cytosol, the normal machinery for MHC class 1 presentation that is present in most cells processes the antigen for presentation in the usual way.

Thus, the results and the relevant controls are evidence of the internalization of MART-1 by PCI, its processing, and its ultimate expression on the cell surface in a form recognized by immune effector cells. It is submitted that Applicants have provided ample evidence to illustrate that the means of performing the claimed invention is described in the specification, and when the process described in the specification is followed, it is effective. It is already known that PCI causes internalization of molecules into the cytosol. The present invention goes further in showing that the internalized molecules are channeled into the antigen processing pathway on their arrival in the cytosol such that they are correctly processed and presented on the cell surface and that hence the method has utility in presenting any peptide that has immune or vaccine potential.

The Examiner comments that Example 2 discloses a cytotoxic cell assay and not cell surface expression. In response, Applicants point out that the invention is directed to a process involving presenting an antigenic molecule or a part thereof on the cell surface, not a process for expressing enzymatic activity on the cell surface. As discussed above, the experiments described in Example 2 and the Declaration show that antigenic fragments of MART-1 were presented on the surface of the cells due to PCI. The claims are not directed to expression of enzyme activity on the cell surface, so there is no requirement to assay for it. Furthermore, one would expect degradation of a peptide or enzyme and processing and expression of only parts of the molecule on the surface. Such parts are likely to be on the order of around 10 amino acids after processing, and as such would be too small to show enzyme activity. The results are therefore entirely consistent with presentation of antigenic parts of MART-1 on the surface of the cells.

The Examiner has stated in the present Office Action not only that neither cell surface expression nor antigenic presentation has been shown, but also that the stimulation of an immune response has not been shown. Applicants have demonstrated with the data of Example 2 and the Declaration that this view is incorrect. Not only do the data show processing and presentation of MART-1 or antigenic parts thereof on the cell surface, but they show cytotoxic T lymphocyte-

induced cell killing of the cells expressing MART-1 or antigenic parts thereof on the cell surface, which is an immune response. Thus, stimulation of an immune response has been shown.

The Examiner also commented that photochemical internalization (PCI) will result in immunosuppression and cell killing, and that PCI and photodynamic therapy (PDT) cannot be distinguished. This view is erroneous, and the Examiner cites no support for it. The extent of cell killing can be manipulated with a high degree of predictability (see the enclosed Rule 132 Declaration).

As described in the Declaration in detail, the range of effects that may be observed when using photochemical techniques may be manipulated depending on the desired result. When cell killing is desirable the procedure may be biased by using, e.g., higher doses of the photosensitizer, longer illumination times, or coupling the procedure with a further cytotoxic molecule which can be internalized into the cell. However, when cell killing is undesirable, i.e., the viability of the cells is required to present an antigen or for expressing a transfected transgene, the procedure should and can be biased towards lower toxicity and reduced cell death. When biased in this way, the less toxic effects of the photosensitizer do not destroy the cell, but merely disrupt its internal compartments sufficiently to allow the transfer of molecules into the cytosol. This is described in more detail on page 4 of the specification, which describes photochemical internalization. See also WO96/07432 (enclosed herewith) which is exclusively concerned with PCI.

The Declaration provides two additional experiments that illustrate the relationship between PCI and PDT, in which the latter results in cell death. It will be noted, particularly from Figure 1, that the result desired is achievable by the appropriate selection of the correct conditions for the experiment. Such selection is entirely routine and since PCI is a known technique, a procedure favoring PCI is readily achievable. The Examples described in the specification achieve PCI without cell death, as will be noted from Example 4, in which the transgene introduced by PCI is expressed by viable cells. In Example 2, the cells remain viable after PCI to allow antigen presentation, and are only killed by the involvement of CTLs.

The Examiner further contends that immunosuppression would occur with PCI. Contrary to the Examiner's assertion, Example 2 shows that presentation occurs and that a normal immune

response results, i.e., recognition and attack by immune effector cells. Furthermore, as cited in the response to the previous Office Action, there are reports in the literature that PDT stimulates immunologic responses. See, for instance, Doucherty at al., J. Natl. Cancer Inst. 90:889-905 (1998), enclosed herewith. Once an antigenic molecule, such as the MART-1 peptide, is presented on the surface of a cell, Applicants submit that there is no reason that proven antigenic peptides would not stimulate an immune response and/or elicit a protective effect. The data of Example 2 and the enclosed Declaration show this to be the case, and the Examiner has not provided any objective evidence that this would not be the case.

Thus, there is no doubt that cells are not necessarily killed using photochemical techniques and that the levels of cells which are killed or which are subject to PCI can be varied by selecting appropriate conditions. As such, Applicants submit that the method of the invention is adequately supported in the specification to satisfy the enablement requirement.

In view of the remarks herein, withdrawal of the rejections of the claims under 35 U.S.C. § 112, first paragraph, is respectfully requested.

The 35 U.S.C. § 112, Second Paragraph, Rejections of the Claims

Claims 6-8 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. These rejections are respectfully traversed.

The Examiner stated in the previous Office Action that the plurals of "lymphocytes, dendritic cells, macrophages and cancer cells" have no antecedent basis in the singular "cell" in claim 6. Claim 6 was amended in the previous response to correct this. The Examiner stated in the previous Office Action that the plural photosensitizing agents have no antecedent basis in the singular photosensitizing agent in the preamble of claim 7. The number agreement is correct in the present claim 7. The Examiner states in the present Office Action that the abbreviations "$TPPS_4$, $TPPS_{2a}$, and $AlPcS_{2a}$" render claim 8 indefinite because they have not been defined in claim 8. Claim 8 has been amended to recite the full names of $TPPS_4$, $TPPS_{2a}$, and $AlPcS_{2a}$. Applicant believes that these claim amendments obviate the rejections. Accordingly, withdrawal of the rejections of claims 6-8 as indefinite under 35 U.S.C. § 112, second paragraph, is

respectfully requested.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612-371-2111) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

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CERTIFICATE UNDER 37 CFR 1.8: The undersigned hereby certifies that this correspondence is being deposited with the United States Postal Service with sufficient postage as first class mail, in an envelope addressed to: Box RCE, Commissioner of Patents, Washington, D.C. 20231, on this 18th day of November, 2002.

PATRICIA A. HULTMAN
Name

Signature